EARLY STAGES IN THE DEVELOPMENT OF SOME FRESH WATER FISHES IN THE PUNJAB.

BY

M. Hamid Khan, M. Sc., F.R.M.S.

With three plates.

Material for study was collected during the breeding seasons of 1921-24 from Departmental farms at Madhopur, Chhenawan, and from different places near the River Beas. The specimens were examined in the living state under a microscope and fixed in different reagents for further study. It is, however, intended to give in these pages a bare outline of the early stages, without going into microscopical details, with a view to help a fish-culturist " to differentiate between the successive ages and stages of growing fish, and between fry of the same age, belonging to species, which may be closely allied zoologically, though far apart economically "(17).

Ophiocephalus marulius is taken as a type of the *Ophiocephalidæ*, *Wallago attu* of the *Siluridæ*, and *Labeo gonius*, *Cirrhina mirgala* of the *Cyprinidæ*.

Ophiocephalus marulius.

Freshly laid eggs were taken from the pond, and kept in a live-car under observation, and their growth and development was from time to time compared with those in the pond.

Outline of the embryo becomes defined within twelve hours. The embryo appears on the left side of the yolk in a belt-like manner. The tail end is swollen, transparent and granular, while the head end is darker. Overnight stage shows the appearance of unpigmented eyes, auditory vesicles and heart. Just before hatching, heart gives off aorta, which passes dorsally to the posterior end to turn back into caudal vein. The latter vessel passes *in toto* into subintestinal vessel just near the attachment of the tail to yolk sac. Subintestinal vessel is formed by the vitelline veins which receive yolk capillaries, and anteriorly open into the heart. Anterior caudal vein brings blood from head, and emerges from behind the auditory vesicle, and breaks up into a set of venous spaces. which together with vitelline network spreads over the surface of the yolk and then goes to the heart (Fig. 1).

The embryonic development goes on rapidly, but depends considerably on the temperature of water, and hatching takes place within 30 to 65 hours. Some eggs hatched out while examining under microscope. The movements of tail ruptures the vitelline membrane, and the embryo appears like an egg with a whip like addendum at one end. Total length is 4·5 m.m., head with yolk sac being 2 m.m. and tail 2·5. The newly hatched larvæ lie on one side, and move their tails now and then, and when they swim they either rotate or spin round. The eyes are colourless, black pigment being confined to the lower portion of the yolk in a semicircular band (Fig. 2.). A few hours later yolk circulation becomes complicated and the caudal vein breaks up into branches, and is joined by the posterior cardinal vein near the bend of the yolk sac (Fig. 3). Blood in the latter vessel flows backward, so that the aortic and cardinal blood circulation is in the same direction. Eight hours after hatching the tail has elongated to 3 m.m. Pigment has appeared in the eyes. Yolk sac circulation forms a complicated system of cappilaries. An invagination, which later on forms anus, is seen in the tail region. Hinder extremity of notochord lies straight and there is amassing of tissue a little before its extremity forming the primordium of the caudal ray system. Heart beats 238 times in a minute. Ten hours later, dorsal aorta and caudal vessel have lengthened backwards, and in the anterior part of the caudal region, close to the posterior extremity of the

yolk sac aorta gives off intersegmental vessels along the anterior face of myotome septa. These vessels run dorsally to form a pair of parietal vessels, which anteriorly fall back into the dorsal aorta just anterior to the auditory vesicle. At this stage pigment becomes scattered and appears in the form of stellate cells in the region of yolk.

Second day hatchings grow from 5·6 mm. to 6 mm. Head becomes distinctly marked off from the yolk sac, and an invagination for the future mouth appears antroventrally (Fig. 3). Eyes are profusely pigmented. Stellate pigmented cells spread round the notochord, multiply, extend to the anterior region of the head, and tend to make the animal opaque. Intersegmental vessels are seen in the head region as well, and their blood circulation is very irregular: in some it flows dorsally to the parietals and in others reversely without any definite alteration or arrangement. Pectoral fins appear as slight bud like out-growths, just behind the auditory vesicles. The posterior part of the embryonic fin shows fine striations.

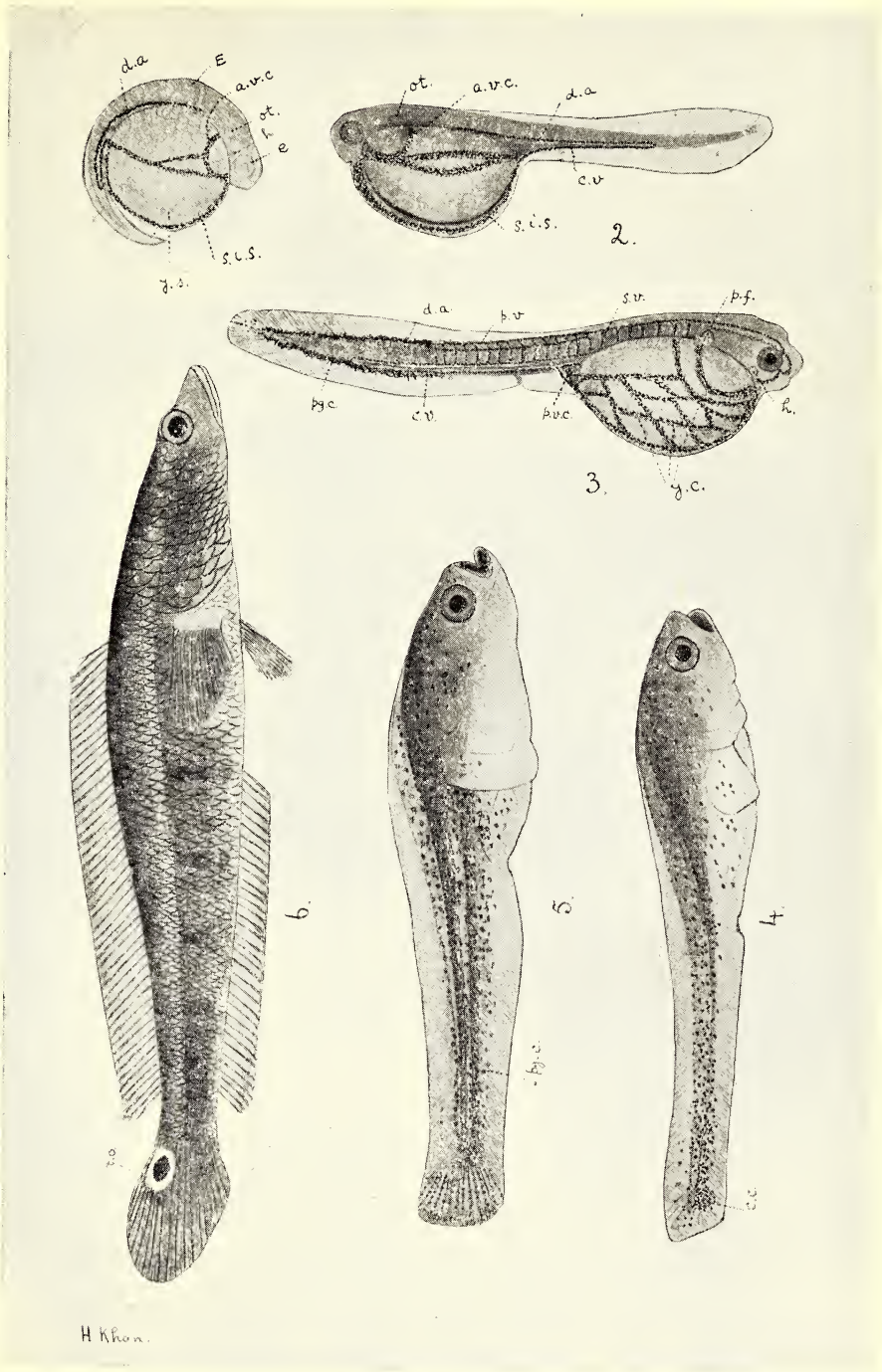
Third day animals measure 7 mm. mouth is open, and respiratory movements have begun. There are no external gills. Lower jaw is well developed, and shows regular rythmic movements. The head is thickened dorsoventrally. The pectoral fins are well developed, and help the animals in their rapid movements. They swim on the surface of water with yolk sac directed up, but when disturbed they go at once to the bottom and remain there for considerable time. Gut is visible as simple tube with muscular rectal walls, but not yet opened posteriorly. Yolk is reduced. Pigment cells are scattered throughout the whole body; and the dorsal and ventral embryonic fins are equally pigmented. Nostrils appear anterior to the eyes.

Next day larvæ measure 7 to 8 mm. Air sacs appear dorsal to the yolk bag, anteriorly, just behind the pectoral fins; and the animals now swim with the yolk sac directed downwards. Pigment cells have become rounded in many places, and lie more on the ventral surface than on the dorsal.

Fifth day shows no increase in length and the animals remain at the bottom for a long time. Spherical pigment cells are arranged in rows on the anterior extremity of the upper and lower jaws, where later on teeth are formed. Alimentary canal is convoluted and opens to the exterior posteriorly, while yolk disappears completely. Black pigment is now visible only in the ventral embryonic fin. Notochord is curved dorsally at its posterior end. Pectoral fins have enlarged and are striated, and have stellate pigment cells (Fig. 4).

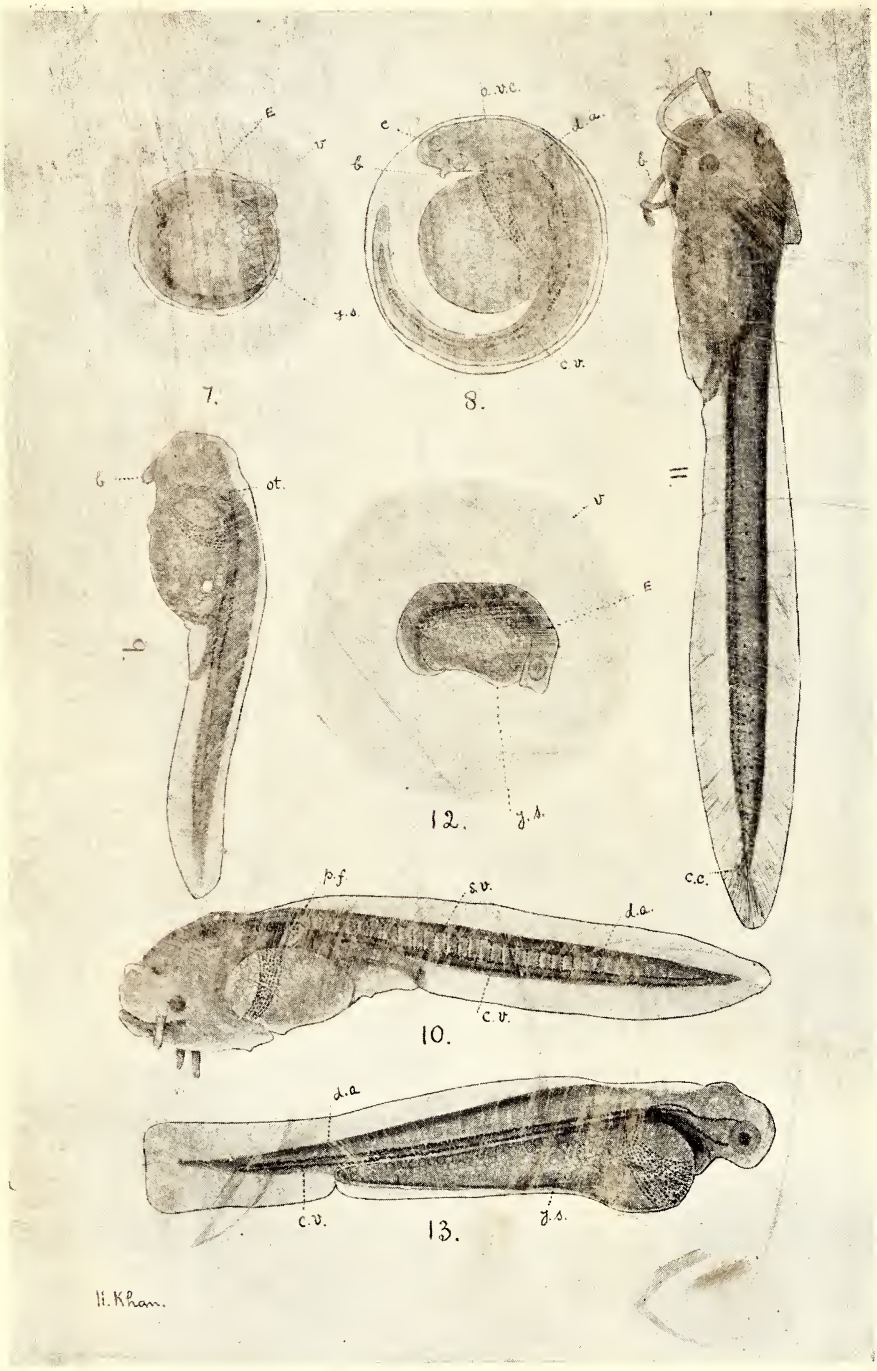
Next two days neither show any increase in length nor any other remarkable change. It may be noted here that since the formation of ailmentary canal and the disappearance of yolk, the growth has not been uniform; but the rate of growth so far has been practically the same between the animals kept in live-car for observation and those in the pond with the parents. The total length in both cases is 8 mm.; head 3 mm.; tail 5 mm. The structure is also the same for the obvious reason that so far they have been getting their food supply mainly from the yolk.

On the eighth day the young ones in the pond, which so far have remained in one place, leave their nest and wander about along with their parents. It is now that a remarkable difference in growth occurs between animals in their natural environments and those kept in confinement. The animals kept in live-car, were fed on minced sheepliver, sieved through a piece of muslin cloth, or with liver-soup and sometimes with infusions of bacteria and mosquito larvæ. They were kept under the same environmental conditions as the fry in the pond, but still they did not show healthy growth. Further structural changes, formation of caudal rays and development of external form of body which took a week in the pond specimens, were completed in three weeks in those kept in captivity and their critical stage appeared with the formation of caudal rays, they became sickly, were attacked with fungus and died in large numbers.



Early stages in the development of some fresh water fishes in the Punjab.

(*For explanation see end of article.*)



Early stages in the development of some fresh water fishes in the Punjab.

(For explanation see end of article.)

On the eighth day circulation in the tail has reached the posterior extremity. The intersegmental vessels are present in the posterior region as well. The pigment cells are round, and are found in a group on the ventral surface of notochord near the posterior end ; and to this mass is given off from aorta a capillary vessel, which turns back and pours its blood in the caudal vein. This is the beginning of caudal capillary system which forms the basis of caudal circulation and marks the development of caudal rays. Next four days show the complication of capillaries, increase of pigment cells (Figs. 14, 15, 16, 17). Pigment cells become arranged in radial rows throughout the caudal fin and the blood vessels too, take the same direction.

Formation of caudal rays depends mainly on the healthy growth of the fry. In warm water, rays first appear on the 10th day, while in cold temperature the growth is retarded. Development of rays begin from the ventral surface and as notochord curves dorsally the rays are drawn towards dorsal surface.

The caudal rays appear as transparent yellowish white strips, in between the radially arranged pigment cells. The loops of blood vessels join together and combine into one. The vessel which carries blood to the ray flows beneath it while the afferent ones lie on its either side. Three basal pieces are also seen.

On the twelfth day (Fig. 5), eleven fully developed caudal rays are visible, segmented at their free ends and striated. Four basal cartilages can be distinctly traced. Caudal circulation is typical (Fig. 18). Just below the posterior end of notochord, the caudal artery branches, and each branch carries bloodtothe finary, and then turns back near the end of the fin, and pours its blood into another system of capillaries, which run on both sides of the fin rays and join to form the caudal vein. The length of body is 10 m.m. On the sixteenth day, caudal fin shows indication of separation from the embryonic fin. The animals have become very active. They come to the surface, exude small air bubbles and then go down and come up, thus causing small whirlpools, which make their presence visible from a distance. Some fry were kept in a dish: They at first came to the surface after 30 to 36 seconds, but later on their breathing became quicker and they were seen rising up after 10 to 20 seconds.

On the 17th day length varies from 15 to 20 mm. Caudal fin is separated off ventrally while dorsally it is still continuous. Eighteenth day marks the end of the larval period by the appearance of ventral or pelvic fins as rudimentary buds in the mid ventral line, a little behind the level of the pectoral fin. The caudal fin separates off completely from the embryonic fin. On the 21st day there are 16 caudal rays; and a yellow band runs dorsally and laterally from the eye to the end of the caudal fin, covering seven caudal fin rays. The fry hide at the bottom when approached, and remain under water for considerable length of time.

Rate of growth of the same lot and of the same year shows considerable difference, and is considerably affected by change in weather, temperature and other environmental conditions. Measurements for the last three years from the same locality do not correspond. For the first four or five weeks the difference in growth is remarkable. Some of the measurements for the last two years are given below:—

	1922.	1923.
1st Week.	8 mm.	8 mm.
2nd Week.	15 mm.	10 mm.
3rd Week.	27 mm.	14 mm.
4th Week.	42 mm.	24 mm.
5th Week.	51 mm.	42 mm.
6th Week.	58 mm.	58 mm.
7th Week.	....	63 mm.
8th Week.	74 mm.	67 mm.
11th Week.	90 mm.	80-83 mm.
12th Week.	98 mm.	99 mm.

Caudal ocellus near the proximal end of the caudal fin appears, in fry ranging from 70 to 90 mm., as a reddish yellow mark, in which later on black oval area becomes visible. Black area measures 1·5× 2 mm. and yellow one 5×3 mm. At this stage fry has white ventral surface, dark grey dorsal and yellowish green sides with one deep yellow lateral band running from the eye to the end of the caudal fin just above the lateral line, and four distinct and two indistinct bluish green transverse bands, running across the body and over the dorsal surface, but interrupted at the lateral band (Fig. 6). Caudal fin is bluish black, pectoral yellow with blue tips, dorsal is dark, grey, while pelvic and anal are yellowish in colour. A few weeks later ventral surface becomes silvery white with slight bluish tinge, while dorsal is of dark greenish blue colour. The lateral band is deep bluish brown and loses itself in the dark back ground of the dorsal surface and becomes less perceptible. Below this band the colour of the body is light yellow mixed with blue and green. There are six to seven transverse bands of bluish green colour. Caudal fin is yellowish tinged with black, dorsal is dark grey and other fins are reddish yellow. The age of the fry at which ocellus appears varies from eight to eleven weeks.

Willey (17) gives his observations on *Ophiocephalus striatus* and S. Raj (15.) briefly describes the development of *O. punctatus* and *O. gachua*, but the life history of *O. marulius* has not yet been studied. Though it does not differ much from its allied species in its general outline, yet the short time taken to finish its larval development, is remarkable. A comparison is therefore made of the corresponding stages of *O. marulius* and *O. striatus*.

Ophiocephalus marulius.

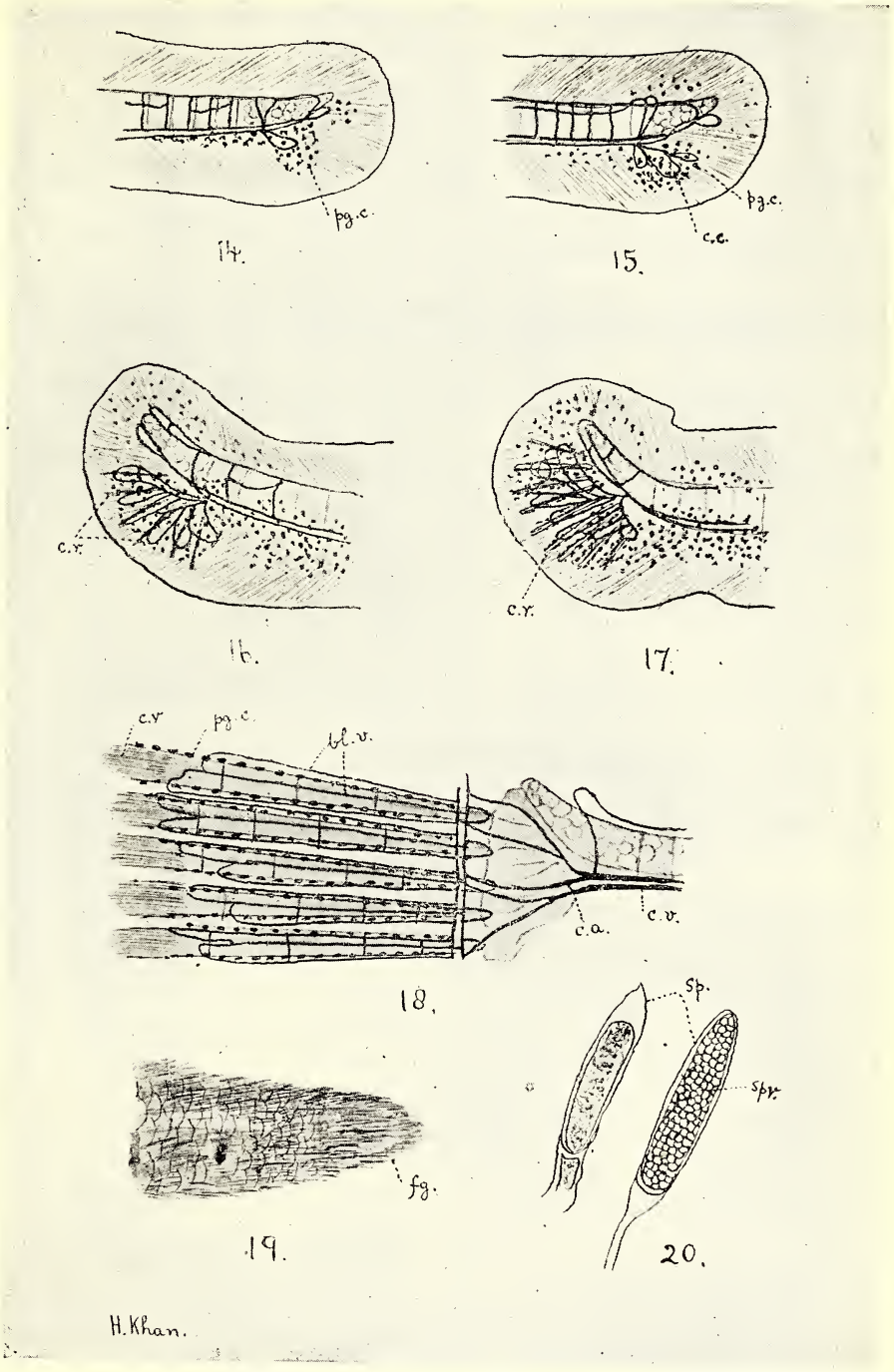
Days after hatching.	Total length.	Principal events.
1st	4·5-5 mm. ..	Yolk sac circulation at first simple becomes complicated. Black pigment cells appear and eyes at first colourless becomes pigmented. Invagination for future anus visible.
2nd	6 mm.	Stellate pigment cells appear. Mouth opens for respiratory movements. Pectoral fins arise. Posterior part of embryonic fin shows striations.
3rd	7 mm.	Pigment scattered equally. Gut visible as simple tube. Caudal vessels are drawn backwards.
4th	7-8 mm. ..	Air sacs formed and embryo swim with yolk bag directed downwards. Pigment cells spherical in some places and lie more on the ventral surface.
5th	7-8 mm. ..	Yolk completely disappears. Black pigment cells on ventral surface only, while dorsal is yellowish. Spherical pigment cells in rows on anterior extremity of jaws. Alimentary canal opens to the exterior posteriorly. Pectoral fin striated and has stellate pigment cells.

Days after hatching.	Total length.	Principal events.
6th	8 mm.	Dorsal aorta and caudal vein have reached the end of notochord.
8th	....	Amassing of pigment cells on ventral surface of caudal fin and beginning of caudal capillary circulation.
9th-11th	....	Arrangement of spherical pigment cells in radial rows, appearance of caudal fin rays and complication of capillary system. Posterior end of notochord is turned up.
12th	8 m.m.-10 mm. ..	Eleven caudal rays jointed and articulated with basal cartilages.
13th-17th ..	10-20 mm. ..	Caudal fin separated off ventrally and movements are very active and the fry rise to surface to take in air.
18th	15-20 mm. ..	Rudiments of ventral fins appear. Caudal fin is separated off completely from the dorsal and anal fins.
19th-21st ..	26 mm.	A yellow band runs on the dorsal surface and ventral one has bluish colour with black tinge.
	Ophiocephalus striatus (Willey).	
1	3·5 mm. ..	Yolk sac circulation established, pigment cells develop their black coloration; pigment begins to appear in the eyes.
2 & 3	4·5-5 mm. ..	Pectoral fins arise, mouth opens and respiratory movements commence.
4	6·75 mm. ..	Larvæ leaving the surface and swimming freely at all levels. Bright yellow spots over eyes.
7	7 mm.	Larvæ swimming and turning in unison at the slightest concussion. Caudal cartilages appear.
12-15	6·75 mm. ..	Posterior end of notochord bends up.
28	8-10 mm. ..	Caudal rays jointed and articulated with the basal cartilages. Larvæ rise to surface to take air.

Days after hatching.	Total length.	Principal events.
37	10 mm. ..	Primordia of dorsal and anal rays.
40	10·25-13 mm. ..	Rudiments of ventral fins appear. Dorsal and anal fins separating from caudal.
63	17 mm.	
73	25 mm.	The fry hide in the mud.

In *Ophiocephalus striatus* " sixty-three days old fry are coloured a soft reddish brown or brown and pink, quite different from the black and yellow of *O. punctatus*. The general colour-effect is dominated by a broad lateral reddish orange band occupying almost the entire height of the myotomes, commencing from the eye on each side, and ending behind with a rounded edge at the base of the caudal fin concentric with the terminal contour of the latter. The iris is golden with a red flush ; there is a bright golden occipital point ; and the basis of the anal fin is dense black along its whole length. The colour of the fry is essentially that which it had acquired at half the size ; and it retains this colour until it has doubled the size, after which the definitive markings begin to appear. Instead of the reddish brown sub-translucent ground colour of *O. striatus* fry, *O. punctatus* fry are characterized by a blackish ground colour, upon which the bright golden yellow bands stand out clear, namely a pair of lateral bands about half the width of *O. striatus* fry bands, occupying the central third of the height of the myotomes and ending behind in a point extending about one-third of the length of the caudal fin into the substance of the fin. Along the length of the back is a golden yellow line running along the basis of the dorsal fin and presenting a more or less distinct interruption in the occipital region in front of the fin at the spot where there is a minute golden speculum in *O. striatus* fry. Besides all this, the *O. punctatus* fry present a clear yellow spot on the snout and do not possess the black basis of the anal fin." (16).

Fungus belonging to *Saprolegniaceæ*, attacks both the adult as well as the fry. It possesses a non-septate branched mycelium, multiplies asexually by club-shaped sporocysts, producing numerous bi-cilliate swarm spores, which emerge, move about in water and finally germinate in another place and produce a new individual of Saprolegnia. Huxley's (5) investigations show that the fungus settles on the portions of the skin of an apparently healthy fish, where there are no scales, and send mycelial or rhizoidal branches through the epidermis into the inner layers of the skin, causing at first local and then general disturbance of the system. Experiment by Rushton (14) on Rainbow trout supporting that of Patterson shows, that Saprolegnia is not the first cause but only a secondary one and only follows a bacillus or attacks on injured surface. In one of our fish tanks owing to the stoppage of water supply for over two months two fish out of thirty-seven survived in 1917. In December 1920 two big fish died in the same tank and they had patches of fungus growth on their body. Inspection of tank showed an overabundance of *spirogyra* and other Algal plants which were immediately removed and no death occurred in the adult fish after that. Fungus again appeared in July 1921 among fry kept in captivity in live-cars. In many cases the fry had their caudal portions discoloured with distinct filamentous outgrowths of Saprolegnia. The live-cars



Early stages in the development of some fresh water fishes in the Punjab.
(*For explanation see end of article.*)